Tetrahedron: Asymmetry Vol. 4, No. 2, pp. 165-168, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

DIRECT GAS CHROMATOGRAPHIC ENANTIOMERIC RESOLUTION OF JUVENILE HORMONES I - III

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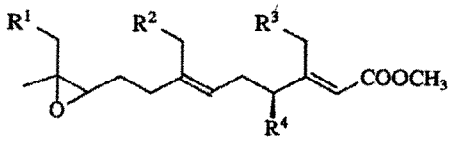
(*Received in UK* 8 *December* 1992; *accepted* 5 *January* 1993)

ABSTRACT: Enantioselective capillary gas chromatography with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin as a chiral stationary phase was used to resolve the enantiomers of juvenile hormones I - III. The assignment of the order of elution of the enantiomers was performed by comparison with synthetic reference compounds.

The development of insects is governed by a considerable variety of hormones. Larval moulting and maturation in adults is regulated most dramatically by the sesquiterpenoid juvenile hormones **1 - 4 (fig. 1).**



1	$R^1 = R^2 = R^3 = R^4 = H$	(JH-III)
2	$R^1 = CH_3$, $R^2 = R^3 = R^4 = H$	(JH-II)
3	$R^1 = R^2 = CH_3$, $R^3 = R^4 = H$	(JH-I)
4	$R^1 = R^2 = R^3 = CH_3$, $R^4 = H$	(JH-0)

Fig. 1. Structures of juvenile hormones 0, I, II and III

Much interest was focused on the biosynthesis and metabolic inactivation of these natural products. Determination of biological half lives of juvenile hormones as well as analysis of binding affinity to carrier proteins and receptors are usually performed with commercially available racemic[1] or with enantiomerically enriched and tritium labelled[2,3] synthetic compounds **1** - **3.** It is now firmly established that the enantiomers of the juvenile hormones differ in their kinetic and thermodynamic behaviour towards esterases and binding proteins[4]. Available methods of stereochemical analysis of minute amounts of radiolabelled juvenile hormones by NMR methods require several chemical derivatization steps which are rather inaccurate and cumbersome for routine analysis[2].

We now report on the gas chromatographic separation of juvenile hormone I - III enantiomers and the assignment of the elution order of the enantiomers by comparison with synthetic optically active reference compounds using capillary columns with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin[5], a new cyclodextrin derivative with great enantioselectivity towards a large variety of molecular structures.

EXPERIMENTAL

Samples

Racemic juvenile hormones I - III were purchased from Sigma Chemie, Deisenhofen (FRG). The (10R) enantiomer of **1**, and the (10R,11S) enantiomers of **2** and **3** were prepared as reported earlier[3].

Gas chromatography

The preparation of the cyclodextrin derivative used in this study was described in detail in a previous publication[5]. A 8 m fused silica capillary was coated with a 1 : 1 mixture (w/w) of heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin and the polysiloxane OV 1701 as described previously[6]. A Carlo Erba Model 2150 AC gas chromatograph with split injector and flame ionization detector was used. Hydrogen with an inlet pressure of 40 kPa served as carrier gas. The chromatograms were recorded with a Merck-Hitachi D-2500 integrator.

RESULTS AND DISCUSSION

Modified cyclodextrins have been used very successfully in recent years as a new generation of chiral stationary phases in gas chromatography for enantiomeric resolution of many racemates, including highly hydrophobic compounds, e.g. saturated hydrocarbons[7]. However, only a few examples of separations of highly volatile oxiranes lacking other functional groups were reported so far[8]. Using the cyclodextrin derivative described here we succeeded in resolving oxiranes carrying epoxy group in 1,2-positions with 6 to 12 carbon atoms and a variety of long-chain epoxydienes, which were identified as pheromones of butterflies[9]. It seems to be a common feature of cyclodextrin derivatives and related to the mechanism of chiral recognition that enantioselectivity decreases rapidly with increasing operation temperature of the gas chromatograph[10]. In order to take advantage of the high selectivity at lower temperature we used a capillary of only 8 m length for the resolution of juvenile hormone enantiomers (**fig. 2**).

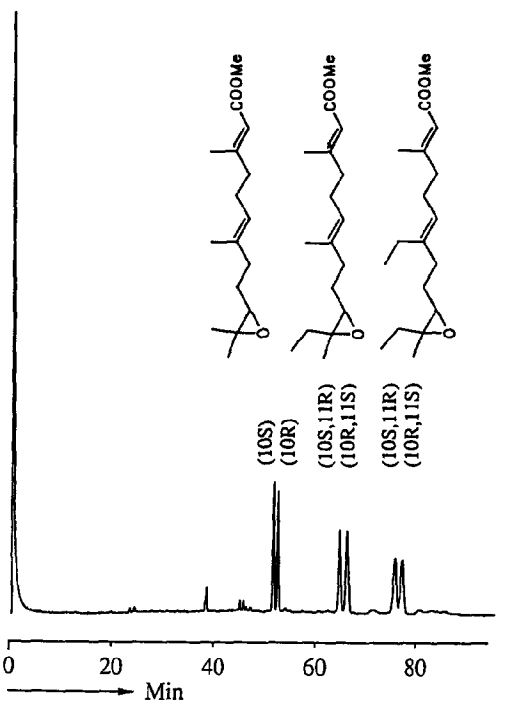


Fig. 2. Gas chromatographic enantiomer separation of juvenile hormones I, II and III. 8 m Fused silica capillary column with heptakis(2,6-di-O-methyl-3-O-pentyl)-β-cyclodextrin in OV 1701 (1 : 1, w/w). Column temperature 80°C, temp. program: 1°C/min to 130°C. Minor peaks between 20 and 50 min are caused by impurities.

The unnatural (10S) and (10S,11R) enantiomers elute from the column before the natural antipodes. This procedure allows rapid determination of enantiomeric proportions of juvenile hormones by any laboratory equipped with gc facilities. Thus, it should now be possible to measure the biological half-life of juvenile hormone enantiomers in insects by a routine procedure with a minimum of sample preparation.

Acknowledgment

We wish to acknowledge the financial support of Deutsche Forschungsgemeinschaft, Fonds der Chemischen Industrie and National Science Foundation (grants DCB8812322 and DCB9119331). We also have to thank Drs. Wai-si Eng, István Ujváry and Mr. Kavish Bhatnagar for providing the optically active juvenile hormones.

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